

Appearance of *Bordetella pertussis* Strains Not Expressing the Vaccine Antigen Pertactin in Finland

Despite extensive vaccinations, resurgence of pertussis has been reported in many countries (9). Recently, emergence of *Bordetella pertussis* isolates not expressing the vaccine antigen pertactin (Prn) or pertussis toxin was described in France, where surveillance of the clinical isolates has been performed since the 1990s and acellular vaccines were introduced in 1998 (1). One hypothesis to explain the emergence of these isolates was previously described (2).

In Finland, DTwP vaccine has been used since 1952 (6). In 2003, an acellular booster vaccine containing detoxified pertussis toxin (PTd), filamentous hemagglutinin (FHA), and Prn (dtaP) (Boostrix; GlaxoSmithKline, Belgium) was introduced for 6-year-old children. In 2005, the DTwP vaccine was replaced by the DTaP vaccine (Pentavac; Sanofi Pasteur MSD, France) administered to children 3, 5, and 12 months of age and booster doses were administered at 4 years of age (DTaP-IPV) (Tetravac; Sanofi Pasteur MSD, France) and 14 years of age (dtaP). Both Sanofi Pasteur MSD vaccines contain PTd and FHA as pertussis components. Furthermore, in 2008 the DTaP vaccine was changed to Infanrix, containing PTd, FHA, and Prn. In Finland, the vaccination coverage has been more than 90% since the 1970s.

We wanted to verify whether *B. pertussis* isolates not expressing Prn appeared in Finland after the introduction of acellular vaccines. We thus developed an indirect enzyme-linked immunosorbent assay (ELISA) for detecting Prn expression of isolates.

Eighty-five strains were included: 76 isolates collected at the Pertussis Reference Laboratory of National Institute for Health and Welfare, Turku, Finland, from 2006 to 2011 (Fig. 1), 7 reference strains with alleles of *prn1* and *prn3* to *prn8*, and 2 French Prn-negative isolates (FR3693 and FR3793) (1). In Finland, the prevalent genotype of the circulating isolates until the 1970s was *prn1*. Since then, *prn2* has been the most frequent genotype (5).

The principles of the indirect ELISA for serotyping of *B. pertussis* were described earlier (4). For detecting of *B. pertussis* Prn expression, monoclonal antibody PeM4 (diluted in phosphate-buffered saline [PBS] at 1:1,000) specific to Prn (National Institute for Public Health and the Environment [RIVM], Bilthoven, the Netherlands) was used. PeM4 has been proven to bind to region 1 of Prn (3). The bacterial culture was performed as described previously (4). The microtiter plate was coated overnight at room temperature with 100 μ l of bacterial suspension and inactivated at 56°C for 1 h, with an optical density (OD) of 0.1 at 620 nm. After 1 h of incubation with the substrate, absorbance was measured at 405 nm with Thermo Multiskan EX (Thermo Scientific, Helsinki, Finland). In each run, the two French Prn-negative isolates were used as negative controls and the purified Prn (2 μ g/ml) as a positive control.

The mean OD values obtained were 0.109 (standard deviation [SD], 0.002) and 0.103 (0.007) for strains FR3693 and FR3793, respectively. The mean OD was 3.266 (0.214) for purified Prn. The OD values of the reference strains *prn1* and *prn3* to *prn8* ranged from 0.57 to 1.88. Of the 76 clinical isolates, 74 were proved to be positive (median OD, 1.30; range, 0.69 to 2.72) and 2 to be negative (Fig. 1). The mean OD values for the two Prn-negative strains

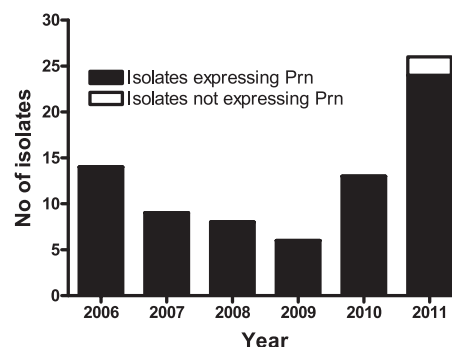


FIG 1 The distribution of 76 Finnish *Bordetella pertussis* isolates tested for Prn expression.

PRCB697 and PRCB698 were 0.121 (0.014) and 0.114 (0.008), respectively.

The PRCB697 strain was isolated from a 10-month-old Finnish girl who had experienced 5 days of paroxysmal cough at the time of nasopharyngeal swabbing. She received two doses of DTaP vaccine according to the Finnish immunization schedule. The PRCB698 strain was isolated from a 4-month-old Finnish boy who had experienced 12 days of paroxysmal cough at the time of nasopharyngeal swabbing. He had received one dose of DTaP vaccine at 3 months of age.

This is the first time that non-Prn-expressing *B. pertussis* isolates have been found in Finland. Both strains were isolated in 2011, 8 years after the acellular booster vaccine was introduced. In Finland, the genotyping for Prn is done by LightCycler PCR combined with gel electrophoresis (6). The method correctly identified the different *prn* alleles. Based on the typing result, the two Finnish Prn-negative isolates harbored *prn1* (data not shown) whereas the French Prn-negative isolates harbored *prn2* (1). The whole *prn* genes of the two isolates were sequenced, and a deletion of 84 bp in Prn signal sequence was found in both isolates, as described in a recent Japanese study (7).

The emergence of Prn-deficient isolates in countries where the acellular vaccines were recently introduced is alarming (1, 7, 8). Our finding clearly stresses the importance of microbial surveillance in order to analyze the evolution and impact of the relevant strains following the introduction of acellular vaccines. Our method was proved to be rapid, reliable, and suitable for monitoring expression of Prn in large numbers of clinical isolates.

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